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Lymphoblast Morphology in Predicting Leukemic Meningeal Relapse With Low Chamber Count and Lymphoblasts

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The diagnostic criteria for meningeal relapse (MR) of acute lymphoblastic leukemia (ALL) are a cerebrospinal fluid (CSF) chamber count of more than five leukocytes per microliter and a cytomorphological evaluation revealing lymphoblasts. A dilemma arises when confronted with a patient with a low CSF white blood cell (WBC) chamber count and lymphoblasts. We utilized a scoring system to review lymphoblast morphology in 12 such patients. A cell was defined as a lymphoblast if it could not be easily categorized as a lymphocyte, monocyte, histiocyte, or granulocyte. Each lymphoblast was scored on four parameters: presence of nucleoli, homogeneous distribution of chromatin, nucleocytoplasmic ratio greater than 75%, and nuclear irregularity. Cells were scored without knowledge of the patients' outcome. Seven patients eventually developed MR by current criteria and five patients never relapsed. The mean lymphoblast scores for patients that did and did not relapse were 2.35 and 1.53, respectively ($P < .001$). The percent of cells scored as lymphoblasts was also significantly higher in patients that relapsed, 36.9% vs. 19.4% ($P = .01$). Our study shows that careful cytomorphologic analysis can predict which patients with low chamber counts and "blasts" on cytocentrifuge examination will progress to meningeal relapse. We recommend reviewing the definition of MR and using a scoring system when confronted with blasts in a low chamber count cerebrospinal fluid specimen. Med. Pediatr. Oncol. 29:98–102, 1997.

Key words: meningeal relapse; lymphoblast morphology; acute lymphoblastic leukemia; diagnosis

INTRODUCTION

The definition of meningeal relapse (MR) in patients with acute lymphoblastic leukemia (ALL) has been a subject of debate and has changed significantly over the years. The current definition employed by the Children's Cancer Group (CCG) is a cerebrospinal fluid (CSF) pleocytosis of more than five leukocytes/μl and unequivocal blasts identified on cytocentrifuge specimen [1]. This definition remains controversial. A dilemma arises when faced with a CSF white blood cell (WBC) chamber count of five or less leukocytes/μl and unequivocal lymphoblasts on cytology. MR has been considered a sign of disease progression and has had an associated poor prognosis [2,3]. Low chamber counts with blasts on cytocentrifuge may represent occult MR in its earliest and perhaps most chemotherapy-sensitive stage. There have been a number of attempts to find an objective marker of MR with only limited success. We developed a cytomorphological scoring system in an attempt to more precisely define MR.

METHODS

A retrospective analysis was performed of children with ALL treated at Children's Hospital Oakland (CHO) from 1987 to the present who had lymphoblasts in the CSF and a low WBC chamber count (<5 leukocytes/μl). Fifteen such patients were identified. Two patients were excluded because their slides were no longer adequate for interpretation and one patient was excluded because he was treated for meningeal relapse despite a low CSF chamber count (<5 leukocytes/μl). None of the patients were receiving weekly intrathecal therapy, had fevers, or recent traumatic lumbar punctures. All of the patients were receiving systemic chemotherapy and were in hematologic remission. The medical records of these patients were reviewed to determine the clinical characteristics, and their CSF cytocentrifuge specimens were obtained for histologic analysis. Some of these patients progressed to MR by current definition, while others never showed signs of disease progression. There were two cytocentrifuge slides for 11 of the 12 patients and they were arranged on the basis of the stain quality. The

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identities of all slides were concealed so that the reviewer's analysis would be blinded to the outcome while scoring cells. Both slides were analyzed. For each slide, up to a total of 100 cells were evaluated, meaning the maximum number of cells evaluated for a given patient would be 200 cells. A potential lymphoblast was defined as a cell that could not be easily identified as a lymphocyte, monocyte, histiocyte, or neutrophil. Such cells were scored (one point each) on four parameters: presence of nucleoli; presence of fine and homogenous distribution of chromatin; nucleocytoplasmic ratio of more than 75%; and nuclear irregularity as defined by a single cleft larger than 50% of the nucleus, or multiple clefts [4].

Figure 1 shows examples of cells that demonstrate these features. After classifying every cell and scoring all the lymphoblasts for each patient, the patients' identities were revealed. The number of abnormal cells, the total number of cells evaluated, the total cell score, and the average cell scores were tabulated. Patients that progressed to MR were compared to those who remained in first complete remission (CR). Of note there were no alterations in subsequent therapy until a patient was definitively diagnosed with meningeal relapse.

Statistical analysis was performed using a Student's t-test after distribution was verified to be close to normal by using the Shapiro-Wilks test. Otherwise, the Wilcoxon rank-sum test was used. Exact logistic regression analysis was used to support the findings generated by the Student's t-test [5]. Bland-Altman plots were used to examine the agreement of the average cell score and the percent of abnormal cells between the two slides [6].

RESULTS

Twelve patients with lymphoblasts on cytocentrifuge specimen and a chamber count of less than five leukocytes/μl between September 1987 and January 1994 were studied. Seven patients eventually progressed to MR and five patients have never shown evidence of disease progression despite close follow-up. Minimum follow-up for patients who remained in CR is more than 36 months. The clinical characteristics including the sex, age at the time of diagnosis, the peripheral blood WBC count at diagnosis, and the CCG risk category were not significantly different between the patients who did and did not develop meningeal relapse (see Tables I and II). Likewise, the median CSF WBC chamber count was similar for patients who did and did not develop meningeal relapse, one leukocyte/μl and 0 leukocytes/μl, respectively. The number of cells examined, the number of cells scored as lymphoblasts, and the total score are summarized in Tables I and II. The mean cell score for patients who eventually developed meningeal relapse was 2.35, while the mean cell score was 1.53 for patients without MR. There was no overlap between the two groups. This difference was statistically significant ($P <$ .001 by the Student's t-test; $P = .003$ by the exact logistic regression).

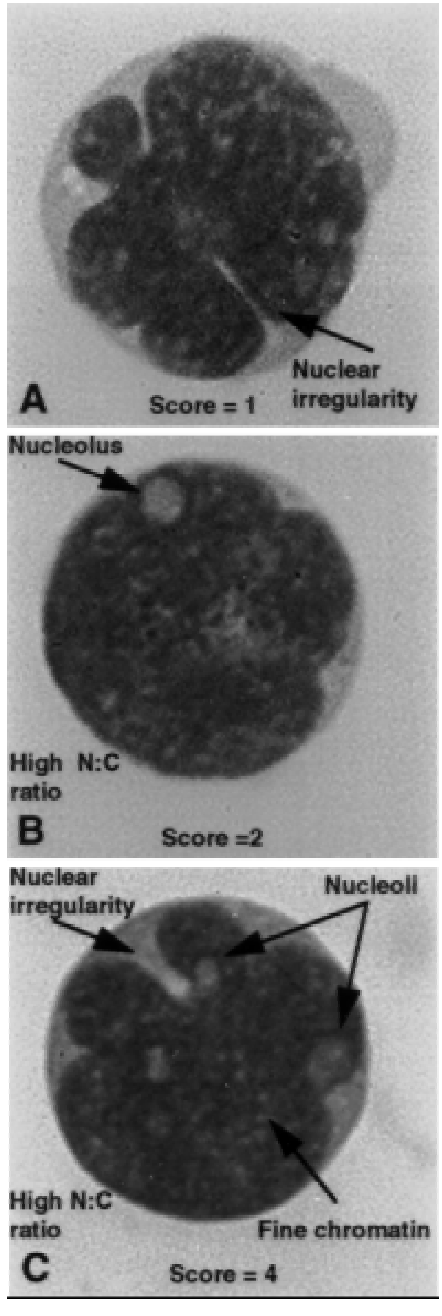

Fig. 1. Shows the four parameters used in the scoring system. **A:** An abnormal cell with nuclear irregularity (score of 1). **B:** An abnormal cell with a high N:C ratio and the presence of a nucleolus (score of 2). **C:** An abnormal cell with all four features (score of 4).

The percent of cells scored as lymphoblasts also showed a statistically significant difference between the two groups. The percent of abnormal cells in the group that progressed was 36.9% and only 19.4% in patients without disease progression ($P = .011$ by the Student's t-test; $P = .008$ by exact logistic regression). Additionally, 83.2% of the cells scored in patients that developed

TABLE I. Analysis of Patients Who Developed Meningeal Relapse

Patient	Sex	Age (years)	WBC at dx (blood)	Risk group	Survival	WBC CSF chamber count	No. of cells examined	No. of cells scored	Percent of cells scored	Total score	Average cell score
1	F	5	3,700	Low	Alive	0	122	30	24.6	77	2.57
2	M	3	3,000	Low	Alive	1	61	22	36.1	58	2.64
3	M	7	21,000	Ave	Dead	1	200	48	24.0	95	1.98
4	F	4	17,500	Ave	Alive	0	88	39	44.3	96	2.46
5	M	4	41,200	Ave	Alive	3	200	115	57.5	260	2.26
6	M	14	28,000	High	Dead	1	200	68	34.0	158	2.32
7	F	5	6,100	Low	Dead	1	192	71	37.0	161	2.27
Average									36.9*		2.35**

*P = .01.

TABLE II. Analysis of Patients Who Did Not Develop Meningeal Relapse

Patient	Sex	Age (years)	WBC at dx (blood)	Risk group (CCG)	Survival	WBC CSF chamber count	No. of cells examined	No. of cells scored	Percent of cells scored	Total score	Average cell score
1	M	2	133,000	High	Alive	0	122	18	14.8	29	1.61
2	M	1	460,000	High	Alive	0	74	16	21.6	28	1.75
3	F	7	3,700	Low	Alive	1	169	42	24.9	76	1.81
4	M	13	2,900	High	Alive	0	30	6	20.0	7	1.17
5	M	2	224,000	High	Alive	0	64	9	14.1	11	1.22
Average									19.4*		1.53**

*P = .01.
**P = .001.

MR had two or more lymphoblast features, while only 46.7% of the scored cells in patients who did not suffer meningeal relapse showed two or more features.

In comparing the two slides, it became evident that the quality of stain can influence the cell score. Because the slides had been arranged in order of staining quality, we cannot reliably compare the average cell score between the slides. It is apparent that the better stained slide leads to a slightly higher cell score in both groups, which can be seen on the Bland-Altman plot (Fig. 2a). As would be predicted, the feature most significantly affected by the quality of the stain was the character of the chromatin (P = .008 by paired t-test). In contrast, nuclear irregularity should not be as influenced by the staining quality and there was no significant difference between the two slides for this variable (P = .216 by paired t-test). The Bland-Altman plot demonstrates that there is no overlap of the average cell score between the two groups.

The percent of abnormal cells present was not influenced by the stain and the Bland-Altman plot shows the reliability of this variable (Fig. 2b). The percent of abnormal cells is not significantly different between the two slides (P = .614 by paired t-test), although there is one case where they differed by 20 percentage points.

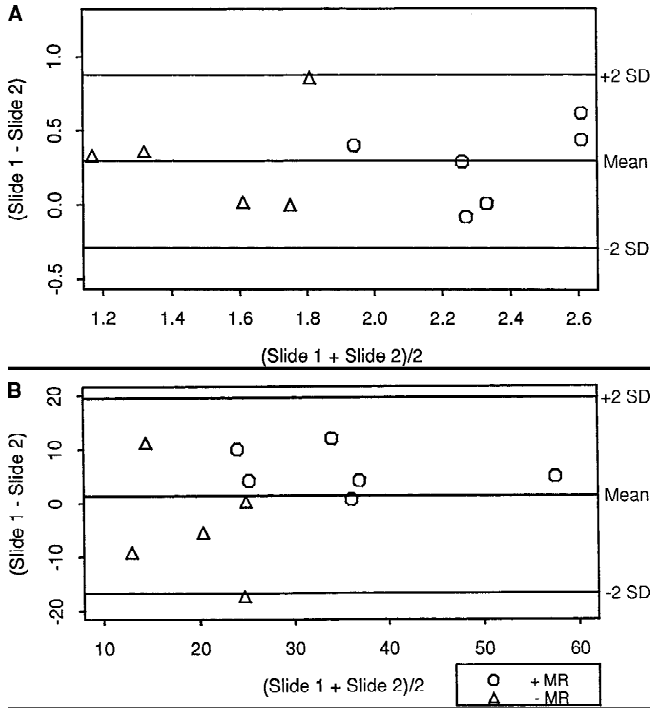

Fig. 2. **a:** Bland-Altman plot analyzing the difference between the two slides for average cell scores. **b:** Bland-Altman plot analyzing the difference between the two slides for percent abnormal cells scored.

DISCUSSION

The diagnostic criteria of MR in ALL has evolved over the years. MR was initially diagnosed in a symptomatic patient with a CSF pleocytosis. Cytocentrifuge technology along with surveillance monitoring allowed for the diagnosis of occult disease. The definition of occult MR continues to require a CSF pleocytosis, although the degree of pleocytosis has been reduced over the past decade. Currently, the Children's Cancer Group (CCG) defines MR as a hemocytometer WBC count (chamber count) of >5 leukocytes/μl and presence of lymphoblasts on a cytocentrifuge specimen [1]. However, this definition remains controversial primarily because of its subjective nature and the dilemma when presented with a low chamber count and lymphoblasts on the cytocentrifuge specimen.

The significance of blasts in the presence of a low chamber count has been the focus of several studies. We previously reported seven patients with low chamber counts demonstrating lymphoblasts on cytocentrifuge months prior to the standard CCG diagnosis of MR [7]. Drewinko et al. [8] showed that children with meningeal leukemia in central nervous system (CNS) remission had subsequent clinical events after blasts were identified despite a normal chamber count. Odom et al. [9] found that 88% patients with meningeal relapse had at least one abnormal low chamber count CSF specimen. More recently, Mahmoud et al. [10,11] claimed that the presence of any lymphoblasts in the CSF of newly diagnosed ALL patients is associated with increased risk of CNS relapse.

Other studies have shown the opposite. McIntosh and Ritchey [12] found that of 15 patients with chamber counts of less than 10 leukocytes/μl and one or more blasts on cytocentrifuge, only three subsequently developed MR. Komp [13] found, after evaluating 1,353 CSF samples from 76 patients with ALL, that three children would have been falsely diagnosed with MR if blasts in any number were used as the diagnostic criterion. A review of patients treated on CCG-105 found the incidence of low chamber counts with blasts to be 0.7% of all CSF specimens evaluated [14]. Patients in whom such an event occurred had earlier relapses, but overall, 5-year disease-free survival was not significantly different from patients without blasts noted on CSF examination. However, none of these studies analyzed the specific morphologic features of the cells that were regarded as lymphoblasts. Additionally, there was no central morphologic review in the CCG-105 report.

The risk in treating patients with low chamber counts and lymphoblasts is the morbidity associated with aggressive MR therapy, including more intensive systemic chemotherapy, frequent intrathecal chemotherapy, and craniospinal radiation. However, by not treating, we may be missing an opportunity to treat MR in its earliest stages, possibly before drug resistance develops, and when the chances of cure are highest.

Defining the lymphoblast and determining which blasts will elude standard therapy are of primary importance. The cytology of lymphoblasts and the diagnostic problems have been described in several studies [15,16]. The main concern is occurrence of a false positive diagnosis of CNS leukemia. Borowitz et al. [16] reviewed 43 CSF specimens from 23 patients with ALL. Of 17 patients that were originally considered positive for leukemia, six proved not to have CNS involvement. However, the author points out in retrospect, that cells from five of the six ''false positives'' were markedly different from the typical lymphoblast morphology. This would suggest that a standardized approach to lymphoblast determination is warranted. One could argue that by eliminating the chamber count currently required for diagnosis of MR, we may increase the number of false positives. If our findings are reproducible, the scoring system should help improve the cytomorphologic determination of CNS lymphoblasts and reduce the number of false positives.

Another important question is whether early therapeutic intervention would influence outcome in patients with low chamber counts and blasts. Fundamental to cancer chemotherapy are the principles that the smaller the tumor burden, the lower the probability of drug resistance, and that temporal delays in therapy may compromise the chance of cure [17]. Theoretically this suggests that outcome would improve, but the only way to answer the question is via a prospective, randomized trial.

In this study, we found the mean cell score of a potential lymphoblast to be 2.35 (range 1.98–2.64) in patients that developed MR by CCG criteria. This was significantly higher than the mean cell score of 1.53 (range 1.17–1.81) in patients that have not developed MR. Although this is a small number of patients, it is interesting that there is no overlap in mean cell scores between those patients going on to MR and those not relapsing. We also found that patients who develop MR have a larger proportion of abnormal cells. Consistent with the previous morphology study, 83.2% of the scored cells had two or more blast features in patients who progressed to MR [4]. In patients that did not develop MR, the majority (53.3%) of the cells scored had only one feature.

Many attempts have been made to find a more objective method of diagnosis [18]. These include various chemical tests such as Beta-2-microglobin, ferritin, and acid phosphatase. Flow cytometry would be useful when an aberrant phenotypic expression is present. Immunophenotyping is difficult with a small number of cells but may become more applicable in the future. Immunofluorescent assay for nuclear TdT has shown some promise when used in conjunction with morphologic evaluation, but this technique requires further study [18–20]. Until a

specific CSF leukemic ''marker'' is found, we will have to rely on careful cytomorphologic analysis for diagnosis.

In designing a prospective study, the average cell score and the percent of abnormal cells should be examined. Individual institutions should analyze these qualities using this scoring system when there are apparent lymphoblasts with a low chamber count in patients with ALL. To avoid issues related to staining artifacts, the same slide should be sent to a central reviewer for an independent histologic analysis using the same scoring system. This will allow the most accurate determination of the reliability of this system for predicting meningeal relapse. Additionally, this should be conducted in parallel with other potential predictive methods such as flow cytometry.

In conclusion, we need a more precise method for diagnosis which will allow for earlier diagnosis and treatment without sacrificing specificity. Using a scoring system such as the one utilized in this study may help reduce the subjective nature of cytomorphologic analysis. We recommend adoption of this scoring system in a large prospective cooperative study to determine its predictive power.

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